SCIENCE LIBRARY

APR 3 1922

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XLII MARCH, 1922 No. 3

CONTENTS

PAGE

ALLEE, W. C. *The Invertebrate Course in the Marine Biological Laboratory.* 99

COPELAND, MANTON. *Ciliary and Muscular Locomotion in the Gastropod Genus Polinices.* 132

UHLENHUTH, E. *The Effect of Iodine and Iodothyrin on the Larvæ of Salamanders, III.* 143

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY, INC.
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XLII *March, 1922* *No. 3*

BIOLOGICAL BULLETIN

THE INVERTEBRATE COURSE IN THE MARINE BIOLOGICAL LABORATORY.

A CONTRIBUTION TO THE TECHNIQUE OF TEACHING ZOÖLOGY.

W. C. ALLEE,

MARINE BIOLOGICAL LABORATORY.

In the thirty-four years of its existence, the course in invertebrate zoölogy, given each summer in the Marine Biological Laboratory at Woods Hole, has undergone considerable evolution. It was founded at the first session of the laboratory in 1888. In the first report of the director, Professor C. O. Whitman, the course is outlined thus:

"The work of instruction, conducted by Professor Van Vleck, was confined chiefly to the study of invertebrate forms, such as the sponges, hydroids, ctenaphors, worms, starfishes, sea urchins, lobsters, corals, etc. An excellent series of mounted preparations added much to the value of instruction. Considerable attention was given to histological technique, and a large amount of valuable material for use in the teaching was collected by each member of the class."

Since that time the course has been in the charge of J. S. Kingsley, specializing in comparative anatomy; H. C. Bumpus, marine zoölogy; James I. Peck, morphology; Ulric Dahlgren, histology; G. A. Drew, biology of molluscs; W. C. Curtis, morphology and regeneration; Caswell Grave, morphology and embryology; and W. C. Allee, ecology and general physiology. Several of these men had served a preliminary term as instructor in the course before taking charge, and this experience made for continuity in its development.

In the earlier years, many of the lectures were given by investigators, not connected with the teaching staff. The whole field of

zoölogy might be considered in this one course, which was designed particularly to meet the need of teachers. In 1893 the work in embryology was taken over in a separate course under the direction of Professor C. O. Whitman, in 1899 Professor Jacques Loeb first offered a special course in comparative physiology, and in 1919 Professor Gary N. Calkins organized the course in protozoölogy; but elementary aspects of these special courses are still retained in the invertebrate course, wherever they are found useful in the study of the subject.

The present type of organization has been in existence since its initiation twenty years ago, in 1901, by Dr. Drew. The conduct of the course as described then by Caswell Grave[1] has seen no radical change, although there has been a gradual evolution away from histological studies towards observations on living animals, in keeping with the zoölogical tendencies of the times.

The instructor in charge of the course bears full responsibility for the selection of the staff, except that the number of staff members is fixed by the laboratory management; there are at present eight instructors and an assistant who acts as preparator and sees to the supply table. The staff is selected from younger instructors or exceptional graduate students and is chosen for teaching as well as research ability, not to mention the physical strength and energy necessary for a long day's teaching and strenuous field trips. An effort is always made to bring these instructors from universities and colleges in different parts of the country in order to have the various types of training represented. The unusual teaching experience is the principal payment for the instructor's labors, although the stipend he receives, together with the accompanying research facilities, is frequently the factor that makes his presence at the laboratory possible.

The work of the staff is divided in open staff meeting with the instructor in charge as chairman, and the general conduct of the course is there discussed and planned. Each member chooses or is assigned some group or groups of animals with which he is especially familiar, or in which he is particularly interested and wishes to work up in the necessary detail. Not only does he

[1] "Course of Study in Invertebrate Zoölogy in the Marine Biological Laboratory at Woods Hole," *Journ. App. Microscopy*, 10, 1482–6.

familiarize himself with the forms to be used in the laboratory and with the general literature of the group, but he also spends time studying the representatives found near Woods Hole so as to be able to identify them in the field. He goes through the special literature in order that he may be able to give intelligent information on the points of unusual interest connected with the phylum, particularly the research problems in which it has played or is playing a part.

The instructor in charge of the course determines who shall be admitted to the course under the rules of the laboratory, supervises field work and has general oversight of the organization. He takes his regular turn in lecture and laboratory along with the other instructors. These are in turn individually responsible for the organization and presentation of their particular groups. Each instructor meets with the men who are to assist him, goes over in detail the work planned, and for the time being has complete charge of instruction. He is aided by "experience" cards which set forth in some detail the results of past methods, but he is not bound by tradition.

The course ends on the second Tuesday in August and begins six weeks earlier. This gives a total of thirty-six working days which are divided as follows:

Course lectures, 29.

Special lectures, two by staff members and a varying number of invited lectures from Laboratory investigators.

Laboratory periods, about 56. This number varies with the number of mid-week field trips taken.

Field excursions, about nine.

Holiday, the Fourth of July.

The formal day begins at nine with an hour's lecture, usually on the group to be studied in the laboratory period following. Although it is difficult to generalize about the lecture methods, it is common with the larger phyla to present schematically an ideal representative of a given group, trace its phylogenetic development, describe in detail special structures, or characteristic physiological activities, and give the differentiations within the group and their relation to the environment in which they are found.

Some attention is directed to important principles that are especially well illustrated in that phylum; relatively little time goes to details of classification.

Blackboard drawings, chart and models are employed in the lecture. In general, we find that students will follow a blackboard drawing made before their eyes, with more interest and understanding, in spite of imperfections in execution, than a description illustrated by the most carefully drawn chart. These drawings are diagrammatic and capable of rapid copying by the class, in preference to exact and detailed reproductions.

Supervised laboratory study follows the lecture for two hours in the morning and again from two o'clock till four in the afternoon; normally three instructors are kept busy, though more may be called in on special occasions; as in the examination of plankton. The instructor in charge usually stays on an hour or more later with the students who wish aid in studying their material more fully. The laboratory is open at all hours, and it is unusual to find it empty between six in the morning and eleven at night. Special assistance is given on request whenever an instructor can be reached.

The students who take this work are required to have had at least one year's laboratory work in college biology and to present a recommendation from their college instructor. The class is limited to 55, and in recent years there has always been more than this number of applications. Qualified students from colleges which regularly support the Marine Biological Laboratory are admitted first; after these are served admission depends on priority of application and on training. The class is drawn mostly from institutions over the eastern half of the United States from Maine to Nebraska and from New Orleans to Minnesota. At least thirty colleges are represented by one or more students each summer. A few apply who present only the minimum requirement, but over half of the class has been graduated from college.

Teachers and graduate students are about equal in number among the college graduates. Older people who desire the course as a matter of intellectual curiosity are always present and welcome. Admission is not knowingly granted to students who use the course as a convenience to work off college conditions.

No text is required, although students are advised to keep at hand some standard reference book, such as Parker and Haswell's "Text-book of Zoölogy," or Lang's "Comparative Anatomy." Pratt's "Manual of Common Invertebrate Animals" is constantly used with the field work. Drew's "Laboratory Manual of Invertebrate Zoölogy," which was originally developed for this course, supplemented by additional mimeographed directions, made out by the instructors from year to year, form the guide for the laboratory work. The Laboratory library is open to the students and they are constantly given references to the literature, but they are more interested in studying the animals at first hand and read comparatively little.

Typically, the subject of Invertebrate Zoölogy is divided on this plan:

Protozoa: Two lectures and laboratory days, followed by a morning spent in collecting fresh-water protozoa from neighboring ponds and an afternoon's work in identifying the forms collected. The laboratory is a compromise between the usual type study and a requirement of a certain number of recognizable drawings. In 1920, for example, *Arcella* was studied the first half day; slides mounted a half hour before use showed active movement. *Ameba, Actinophrys,* and *Actinosphærium* were also demonstrated. Almost every one has studied *Paramecium,* but by feeding the animals with hay bacteria stained with neutral red, the student, in the course of watching the feeding activity and the subsequent change of the food vacuole from acid to alkaline, unconsciously reviews many points of former observation, to his considerable advantage. Hypotrichs are always in reserve for this work.

The second morning was occupied with *Euglena* and *Volvox,* with *Ophridium,* a green colonial vorticilid, and the gregarine, *Schizocystis,* from *Phascolosoma,* in reserve. The afternoon was spent on the flagellates parasitic in termites.[1] These form an easily obtainable and fascinating group and it was hard to divert

[1] Leidy, Jos., "Parasites of the Termites," *Jour. Acad. Sci. Phila.,* VIII., pp. 425–447 and plates, 1881. Porter, J. F., *Bull. Mus. Comp. Zoöl.,* XXXIII., 1897. Kofoid and Swezy, Univ. of Calif. Pub. Zoöl., XX., 1919.

the students' attention to *Ephelota* and *Acineta*, the Suctorians found attached to old colonies of *Obelia*.

In the identification of protozoa found in the field, it has been found desirable to ask the student to classify as far as possible; after the order, identification is given or checked by the instructor.

Porifera.—One day, lecture and laboratory. In addition to the more usual study of *Leucosolenia* and *Grantia*, students perform H. V. Wilson's experiment[1] on the dissociation of *Microcione* cells. After squeezing these through miller's bolting cloth, identifying the cells and watching early stages of the plasmodium formation, the slides are put out in a live car under the wharf and taken in for inspection at the end of the course almost six weeks later. Living *Grantia* are sectioned and choanocytes and flagella studied in the normal condition. In order to make this successful the material is not exposed to the air even momentarily until it is sectioned, and then for as short time as possible; the *Grantia* is freshly collected and the razors very sharp.

Cœlenterata.—Four days, lecture and laboratory. In 1919 and 1920 the work on Cœlenterata was begun by starting experiments on regeneration in *Tubularia*. The attention of the class had previously been called to the recent paper of Garcia-Banus and the conflict between that report and the commonly accepted ideas on *Tubularia* regeneration.[2] Data were collected each day under the personal supervision of some staff member who inspected each piece of *Tubularia* and final results were tabulated on the blackboard. The results for 1919 are quoted by Hyman[3] (p. 358) and were supported by the data of 1920 and both confirm the results obtained by Child[4] and Hyman rather than those of Garcia-Banus. Needless to say, class interest in this experiment was intense, particularly among the more advanced students.

The work on the Hydrozoa includes time spent on the structure

[1] Wilson, H. V., "Development of Sponges from Dissociated Tissue Cells," *Bull. U. S. Bureau of Fisheries*, 30, 1910.

[2] "Is the Theory of Axial Gradient in the Regeneration of *Tubularia* Supported by Facts?" *Jour. Exp. Zoöl.*, 26, pp. 265–275.

[3] "The Axial Gradients in Hydrozoa. III. Experiments on the Gradient of *Tubularia*," BIOL. BULL., 38, p. 358.

[4] "An Analysis of Form Regulation in *Tubularia*," *Arch. f. Entw'lungsmech.*, 24, pp. 1–28.

of *Tubularia* and *Obelia*. These are normally fruiting at the time studied. A morning in which the student sees fruiting *Tubularia* and watches *Actinulæ* emerge and crawl around, added to an afternoon studying *Obelia* which are shedding *Eucope*, makes a day's work that the instructors, at least, never forget. To insure a plentiful supply of *Eucope* or *Actinulæ*, the material is brought into the laboratory a few hours before being studied and allowed to stand in a shallow dish in fresh sea water. The *Eucope* come out more readily if placed in the sun and are to be found on the sunny side of the dish.

Fruiting *Eudendrium*, *Clava*, *Hydractinia*, *Schizotricha* and *Sertularia* are also available for observation and study. In 1921 following a mild winter and an early spring, we could obtain fruiting *Bougainvillia*, *Campanularia* and *Pennaria*. *Gonionemus* is studied alive in some detail and living *Physalia* is usually on hand at some time in the six weeks.

In *Hydractinia* the striking *dactylozooids* are readily demonstrated by removing the hermit crab on whose foster shell they grow and stimulating one edge of the colony by scratching with a needle. Under a low-power lens the wave of stimulus can be followed across the colony as the *dactylozooids* strike. These "persons" of the colony are more abundant near the mouth of the shell than elsewhere. In *Gonionemus*, the lithocysts are readily studied by cutting a V-shaped piece from the margin and mounting aboral side up under the high power.

The work on the development of *Aurelia* is on preserved and stained material, although there are usually living *Aurelia* in the laboratory, and sometimes enough of it to supply the class. The canal system is here best demonstrated by injecting it with air, which is easily done even in preserved specimens.

With *Metridium* a half day is spent in repeating Parker's[1] experiments on feeding reactions and an opportunity is given for dissecting recently preserved specmens that have not yet lost all their color. Prepared sections showing relations of the mesenteries are present for special study and living and preserved *Astrangia* is at hand for those who have done the other work. The

[1] "The Reactions of *Metridium* to Food and Other Substances," *Bull. Mus. Comp. Zoöl. Harvard*, 29, 1896.

last half of the day of the Cœlenterate group is spent on *Pleurobrachia* which Mr. G. M. Gray preserves in life-like transparency. These are studied in the original preservative, rather than in water.

Platyhelminthes.—Three days, lecture and laboratory. This study begins with locomotion and feeding reactions of fresh-water planaria, and regeneration experiments are started. The animals are kept in pond water. We introduce the class to living *Syncœlidium* or *Bdelloura* from the gill books of *Limulus*. These have lost their pigment and the structure even to flame cells, is visible. Cross sections have been used of late years to give a better demonstration of the relation of the proboscis to other structures. The Cestodes are studied with living *Crossobothrium* when sand shark spiral valves are available; if these are not to be had, *Rhyncobothrium* and *Calliobothrium*[1] from the spiral valve of the dogfish are substituted. Flame cells are readily seen in the scolex of any of these. The encysted scolex of *Otobothrium*[2] found in the muscles along the vertebrae of the butterfish gives an example of the everted pleurocerous stage characteristic of the Tetrabothridia. In 1921 a study of embryonic stages of *Rhyncobothrium* was added to supplement the work on the encysted scolex of *Otobothrium*. The following stages were studied: (1) The freshly laid eggs; (2) eggs in which the hexacanth embryos were developed; (3) emergence of embryos from egg case; (4) free swimming hexacanth embryos with ciliated embryophore; (5) embryo from which the embryophore has been torn off. Directions for obtaining this material as worked out by Dr. Bowen are given in the appendix (1).

A half day is spent on *Tetrastema*, the little nemertean from pilings. These are secured by allowing scrapings taken about 6 A.M. to stand for three hours in fairly deep glass jars filled with sea water; the nemerteans can be picked up with a pipette and a lighted candle held by the jar just below the water level makes them more easily recognized. Scrapings spoil if allowed to stand

[1] Linton, E., "Notes on the Entozoa of Marine Fishes of New England," U. S. Comm. of Fish and Fisheries, Comm. Report, 1886 and 1887.

[2] Linton, E., "A Cestode Parasite in the Flesh of the Butterfish," *Bull. Bur. of Fisheries*, 26, 1906.

over night. If time permits a half day is also spent on the common rotifers since no other place is available for this neglected group.

Echinoderma.—Four days, lectures and laboratory. At present this work is introduced by a day's study of maturation, fertilization, cleavage and development stages of the starfish egg, with sea-urchin and sand-dollar material in reserve in case of need. Approximate schedules for different stages in the development of *Asterias, Arbacia* and *Echinarachnias,* as worked out by Miss Christianna Smith, are given in the appendix (II.). Preparation for this day's work takes much time and careful cooperation with Mr. Gray in the matter of securing plenty of ripe starfish, since this is near the end of their breeding season.

Work on the adult starfish begins with a study of the activities of the living animal, which includes an annual repetition of Cole's experiments on righting reactions and locomotion;[1] in spite of diffuse lighting and class technique, these have on the whole supported Cole's contention that there is evidence of physiological asymmetry in the starfish. The free cells in the cœlomic fluid are also studied. We obtain these easily by hanging the starfish up by one arm, catching a drop of the fluid as it escapes and examining immediately before aggregation takes place. Circulation in the cœlomic fluid and the dermal branchiæ is demonstrated by means of carmine injection.

The sea-urchin takes more than a day for dissection even though little time is spent on the arrangement of plates in the test. Aristotle's lantern is studied in detail and the genital rachis in *Strongylocentrotus* is dissected. For this work specimens preserved in alcohol are used. The dissection is made by picking off the skeleton in the equatorial region, leaving one ray intact. The exposure is gradually extended to the dorsal region, which is held up as long as desired by the part of the shell still intact. This gives better results than the classical method of dissection.

Ophioderma and *Leptosynapta* are present in reserve for those who have done the regular work. The latter are kept from the

[1] "Experiments on Coördination and Righting in the Starfish," BIOL. BULL., 24, 1913. "Direction of Locomotion in the Starfish," *Jour. Exp. Zoöl.*, 14, 1913.

time they are collected in separate bottles placed in a larger dish of running sea-water to prevent fragmentation. We usually study the swimming motions of *Ophioderma*.[1]

Living representatives of all available echinoderms are kept in aquaria in the center of the laboratory. Some of these contain starfish and mussels and give the starfish an opportunity to demonstrate their feeding methods. *Thyone* are allowed to bury themselves in muck and show their usual feeding reactions and method of respiration.

The last half day is spent on a dissection of narcotized *Thyone*. For this work freshly collected specimens are best. These are injected about an hour before use with about 15 cc. of chloretone solution recently saturated when hot. After a short time the tentacles can be forced out by gentle manipulation and the narcotization holds during the dissection in sea water. It is essential that the chloretone solution should be as strong as possible. There is a great difference between these narcotized animals and the usual formalin faded specimens.

Annelida.—Three days, lecture and laboratory. *Nereis virens* living, preserved, or narcotized in alcoholic sea water, forms the entering wedge for this group. Cleared transverse sections of whole segments are also used to show general relations and especially the parapodia. *Arenicola,* freshly narcotized, are dissected for the blood system and for the nephridia, following directions based on Ashworth's monograph.[2] Sometimes we dissect *Amphitrite* (see appendix III.) and a large collection of other worms is present in the laboratory. Usually *Lepidonotus* is studied for external features, and of recent years a half day has been spent in classifying local annelids, using Pratt's key. Phosphorescence can be successfully demonstrated in *Chætopterus* by taking the animals to the dark room at night, and the class collects swarming *Nereis limbata* when moon and weather permit. The last half day is normally spent in a dissection of the common sipunculid, *Phascolosoma,* freshly narcotized in alcoholic sea water.

[1] Grave, Caswell, "*Ophiura* (*Ophioderma*) *brevispina*," Mem. Biol. Lab. Johns Hopkins Univ., 4, 1900.

[2] Ashworth, J. H., Liverpool Marine Biology Committee Memoirs, XI., 1904.

This laboratory work has sometimes been introduced by a study of the 48-hour *Hydroides* trochophores. The primitive structures can be easily made out. These are followed by 5–7 day larvæ showing the greater development of the tail region.

Bryozoa.—One day, lecture and laboratory. Living *Bugula* furnishes the main attraction, and some time is spent on the encrusting forms.

Crustacea.—Three days, lecture and laboratory. The treatment of this phylum has undergone more changes than that of any other group in my eight years on the course. At one time a week was spent in detailed study of their homologies.

We give one afternoon of the three days now allotted them to studying tow, which contains a large number of larval crustacean stages at this season of the year, but is examined for other animals as well.

The lobster is the introductory animal, with the blue crab as alternate for those who have already dissected the lobster or the crayfish carefully. These are injected and hardened (see appendix IV.) in formalin for two weeks before using. One day is given to this dissection, with an extension of time for those who need it. More time could well be spent here but it is better used elsewhere in this crowded course. Some study is made of the usual Entomostraca, such as *Cyclops* and *Argulus* and a half day is spent in working out appendages of Malacostracan forms, which are recorded as shown in appendix V. While on the chart rack the information given in appendix VI. is displayed to aid the students in making comparisons readily among the forms studied.

The work is finished by a half day spent in dissecting *Lepas* with *Balanus* present for comparisons. Among the interesting side lights that have been presented from time to time the following have been especially successful:

Argulus placed in finger bowls with *Fundulus* attach themselves to the fish and change color as the *Fundulus* change from being placed on different backgrounds. This is difficult because of the time required to accumulate enough *Argulus*.

Blood coagulation is shown in *Oniscus* by snipping off the filamentous tip of the antennæ. A drop of blood is caught on the

coverslip and by staining in methyl green or aqueous iodine, a cell-agglutinating type of blood-clotting may be observed.[1]

Autotomy is usually demonstrated in *Uca*, the fiddler crab, by crushing the distal end of one or more of the legs with a pair of forceps. Chromatophore activity is well shown in *Palæmonetes*.

Limulus.—One day, lecture and laboratory. We formerly gave a half day to the external anatomy of *Limulus*, using small specimens. At present in addition to the time on the external anatomy and feeding experiments, a half day that usually stretches well on into the night is spent in dissection. *Limulus* is particularly interesting to us as a persistent type, because of its use in phylogenetic theory and because it is a characteristic and common large invertebrate of Woods Hole. The dissection directions have been worked out in the main by Dr. R. H. Bowen and are given in full as Appendix VII, since I know of no other directions in print.

Mollusca.—Five days laboratory, four lectures. This group is introduced by work on *Chætopleura*, which may be mounted on glass slides if put under water, and may then be readily studied on both dorsal and ventral sides. The principal study of the group centers about *Venus* (or *Unio*), *Busycon* and *Loligo*. Usually one instructor is in charge of the earlier part of the work and another has the Cephalopoda.

In the Pelecypoda, comparative studies are made of *Pecten*, *Mytilus*, *Mya*, *Ensis* and *Ostrea*, with a drawing of one form; and living and preserved slides are studied in *Venus*, *Mytilus* and *Ostrea*. The gill current can best be seen in the latter on account of the large large size of the palps on which ciliary currents can be seen. In the opening dissection, the blood system shows much better if injected through the ventricles.

The work in this group also includes study in locomotion of *Ensis*, *Yoldia*, and *Solemya*, and egg laying, fertilization and cleavage in *Cumingia*.

The gasteropod work is limited to *Busycon* (*Sycotypus*, *Fulgur*). These are killed and injected a week before using in order that they may be hardened sufficiently to get rid of slime

[1] Tait, *Jour. Mar. Biol. Assn.*, 1911.

without being too hard for satisfactory dissection. One and a half days should be spent on this dissection, although the familiar device of allowing half the class to work out and demonstrate the circulatory system while the others reciprocate with the nervous system, disposes of the dissection in a day.

Cephalopod study is limited to the common squid, *Loligo.* Living specimens are in the students' aquaria and in the central tanks through the week; egg laying frequently occurs. The most spectacular demonstration of the course comes when the living squid are injected through the buccal sinus for venous system and through the heart for arterial, and studied immediately in salt water. Almost the whole system can be made out without cutting. The better prepared students make the complete dissection of the spermatophoric organ, for which directions are found in the third additon of Drew's Manual. It is obvious that in this work, as on *Busycon,* there is not time for finished dissections and drawings, but it is surprising how much good work can be done in the concentrated effort of a long day, especially if drawings of external features be largely omitted.

Chordata.—Two days, lecture and laboratory. Chordate studies are limited to the Hemi- and Urochordata. The work begins with a study of external features of *Dolichoglossus.* These may be collected Saturday afternoon and kept until Monday in good condition if placed in separate bottles over which a good supply of water flows. The remainder of the first day is spent largely on *Molgula,* living and preserved.

Perophora is used to demonstrate the circultion and the modification found in a stolon type. Young *Botryllus,* that are still transparent, are also present in the laboratory in reserve. *Amaræcium* tadpoles, alive and stained, take up the last morning of the course. The last afternoon is spent in working out some form, such as the adult *Amaræcium,* with no help other than that to be found in texts and laboratory guides. The exercise is not an examination; it counts no more in our estimation of the ability of students than that done on any other half day. It gives the student an opportunity to orient himself under conditions less favorable to rapid work than those that prevailed in the preceding

part of the course; and it holds the attention of the class completely even past the official end of laboratory work.

No attempt is made to put the entire class through a fixed schedule of laboratory work; even where everyone is working on the same material, as much as possible of the method of attack and particularly of the method of recording observations is left to the individual. Students who desire space in the laboratory and access to laboratory material but who have their own plan of work they desire to follow, are welcome as a matter of course.

Field Work.—The field work is one of the most important aspects of the entire course; even the anatomy cannot be clearly understood until the animal has been seen in its native haunts. Gross collecting methods are avoided as far as possible and the student is urged to observe carefully while collecting where the animals are found and what they are doing.

The field schedule for 1921 follows:

July 2, Saturday:

Collecting Protozoa 10:00–12:30.

Examination of material in laboratory, 2:00–4:00.

July 6, Wednesday:

Crane's Wharf. Start 2:00; low tide, 3:20; return about 4:00.

July 9, Saturday:

Vineyard Haven Wharf. Start 8:45; low tide 3:20; return about 2:00.

Examination of collection in laboratory, 2:00–3:00.

July 16, Saturday:

Hadley Harbor (Northeast Gutter Flats). Start 10:00; low tide 11:55; return about 3:30.

July 20, Wednesday:

Hadley Harbor (Gutter Rocks). Start 1:45; low tide, 2:23; return about 4:15.

July 23, Saturday:

Dredging, Vineyard Sound.

Section 1, teams 1–4 start 10:00; return about 12:00.

Section 2, teams 5–8 start 2:00; return about 4:00.

July 28, Thursday:

Study of "Tow" in laboratory, 2:00–4:00.

July 30, Saturday:

North Falmouth Flats. Start 8:45; low tide, 9:35; return about 3:00.

August 4, Thursday:

Kettle Cove, Rocks and Flats. Start 1:45; low tide, 2:33; return about 5:00.

August 6, Saturday:

Picnic. (1921, Penikese.)

Practically the same field organization has been used for the last eight years. The class is divided into as many teams as there are instructors; the team has the same members throughout the course, but the instructors rotate so that each team has the benefit of three different instructors in the field. In each team one student is recorder for the day; others have various assignments depending on the trip. If this be to a flat, two have shovels, one a bucket and crystallization dish, one a *Cumingia* sieve, and the rest carry collecting kits with bottles and jars, forceps and lenses. All are mentally and physically prepared to get wet and dirty.

The Protozoa field trip follows the Protozoa work in the laboratory. While the class is studying Cœlenterates and sponges, two trips are made to wharf piles. Collecting is done from small boats, and glass-bottomed buckets and scrape nets are an important part of the equipment. The trips to flats and rock gutters, and the dredging both depend for their sequence on the tides. It is a general rule never to take a trip in the morning and attempt regular class work in the afternoon. We have found that the students are too tired or diverted to settle down to class dissection. They are interested in working over the specimens collected on the trip and this informal study has become more common of recent years.

At the beginning of the course the student is supplied with a check list of the animals taken by the class in the last ten years. It is suggested but not required, that these be made the basis of class records of experience for the season, so that the student obtains a permanent record of the habitat distribution found by the class collecting that year.

The recorders are furnished with a board bearing a list of the

animals expected from the habitats in the locality to be studied. This is arranged so that an animal may readily be recorded from all the habitats in which it may be found. So far as possible the instructor attempts to teach the students in the field classification down to genus. Species identification is given the student only when of special interest or, in the case of the *Nassas* and *Littorinis,* necessary. The instructor calls attention to interesting facts in the distribution, and other points calculated to make the student more familiar with the animal under consideration. Each instructor is supposed to have a wide knowledge of the group on which he lectures, and doubtful specimens are referred to him for identification. Animals that cannot be named in the field go into the "question mark" bottle for later reference.

On returning to the laboratory a complete list of all the animals found by the class is posted on the board. The list in Appendix VIII. gives the result of one day's find on the North Falmouth Flats in 1920. The list is entirely typical and shorter than the lists frequently reported. No one team found all of these; the individual teams listed from 50 to 96 species, depending on the ability of the team members, the experience of the instructor and the run of collecting luck. It is on record that twice teams have collected and their instructor identified more than a hundred species in one afternoon; but usually there is no effort made to obtain mere numbers of forms. The emphasis is rather on the characteristic animals in the different habitats studied.

While students may make collections and spend considerable time over detailed classification if they desire to do so, they are not urged in this direction. Neither is there any attempt to have students write up accounts of the field trips; even the keeping of records of the animals found is optional. This is in keeping with the general policy of instruction, that as much as possible must be left to the initiative of the student. Each year there are a few students with especial interest in field studies who devote extra time to them; but it is surprising how well the average student learns to recognize and name characteristic animals with no insistence on the part of his instructors.

After one of the early trips all animals taken are demonstrated

in separate finger bowls in order that a named collection of living animals be at hand for closer inspection than is possible in the field; later only the rarer animals are demonstrated. The course possesses a named collection of the common molluscs of the region and is accumulating preserved collections of other groups.

With the exception of the afternoons on Plankton and Protozoa, little attempt is made to identify for class purposes the nearly microscopic animals that may be taken, since it is the purpose of the trips to acquaint the students with the larger, more outstanding members of the local fauna. Doubtless we pass over more species than are recorded. The field collecting has given a number of new records for this well-worked region, has yielded material for one study of regional and seasonal distribution,[1] and is the basis for a study of the littoral ecology of Woods Hole now in preparation.

In earlier days and again for the last eight years a catalogue record of species taken has been kept and is on record as a part of the library of the Laboratory. The person in charge of this compilation is frequently called on to suggest to investigators available material suitable for certain investigations, or the whereabouts of animals not usually collected by the supply department.

Near the end of the course the field work is summarized in an evening lecture on the ecology of the region, illustrated by slides obtained from the American Museum of Natural History and by projections of living animals.

The students have the advantage of the bi-weekly evening lectures maintained by the Laboratory; in addition, special lectures directly related to the work of the course are arranged for the benefit of the class. These are usually given on Saturday morning before the field trip for the day, or in the evening, and they not only convey the information, but introduce the class to some of the interesting personalities among the research group. In 1920 these special lectures were given:

"Some Aspects of Physical Organization of Protoplasm," by Robert Chambers.

[1] Allee, W. C., "Note on Animal Distribution Following a Hard Winter," BIOL. BULL., 36, pp. 96–104.

"Observations on Egg-laying in the Trematode, *Epobdella*," by Edwin Linton.
"The Elementary Nervous System," by G. H. Parker.
"Metamorphosis in the Echinoderms," by Caswell Grave.
"Zoölogical Research," by C. E. McClung.

The Laboratory gives no credit for work done in its courses, nor does it keep formal record books. A student so desiring may receive a certificate stating that the work of the course has been satisfactorily completed and may then cash this in at his college for undergraduate, and in most institutions, for graduate credit (*e.g.*, Chicago, Wisconsin, Cornell). All colleges do not allow the same amount of credit; the usual practice is to give from 5 to 6 semester hours' credit for the work of the course. Any student may have his laboratory notes inspected by the instructor in charge of the group, and all persons asking for certificates must submit their notes for such inspection.

The course outlined is sufficiently different from the ordinary college course, both in its method of presentation and its effect upon students, to arouse constant curiosity as to its essential points of variation. At Woods Hole there are unusual temptations to sight-seeing, boating and bathing, but the attitude of the class is anything but the summer-vacation type that prevails in many summer schools.

The great efficiency and speed attained and held by the class through the six weeks must be ascribed in some degree to the presence of three trained teachers, the instructor in charge and the two assisting members of the staff, who are always at hand in the laboratory when needed; though they give as little oral instruction as possible, so that the student is a more independent worker at the end of the course than he was at the beginning.

I am inclined to think that the intensive work which the students accomplish is due in part to the very speed with which ground is covered. It appeals to the imagination of the student to look back at the end of ten days over the Protozoa, the sponges and cœlenterates, with three field trips extra. Further, he has the advantage of no other study to distract his mind. Through the winter his attention must ordinarily be stretched over three

to six diverse subjects; these six weeks at Woods Hole belong to zoölogy alone.

He has before him the constantly shifting panorama of the animal kingdom, and each animal group is presented by a new instructor with a fresh point of view and a different method of procedure. It is an interesting sight to watch the class when fagged near the end of the course, rise to the stimulus presented by an instructor with a new way of looking at things in general and this especial group of animals in particular.

Another potent cause for sustained interest is the excellent supply of living or recently preserved animals which Mr. Gray, of the supply department, keeps in the laboratory. Up to the limit of his working ability, the student will not let this material go to waste.

The intercollegiate character of the class personnel gives stimulus to high-grade work. If undergraduate, the students are usually the pick of the department sending them; if graduate students, they are at least serious-minded. Many of them feel that they are personal representatives of their college and more especially of their home department of zoölogy and they take pride in bringing credit to their training.

Finally, there is not the usual reserve between instructors and instructed. To a great extent the general attitude is that of two groups of people, both recognizing that they are fellow-seekers after information, but one, due to greater experience, obliged to confess complete ignorance less frequently than the other group. Even if he desire to do so, the instructor cannot keep a dogmatic attitude in the face of eight students digging all sorts of animals from the sand and mud and asking questions which a dozen specialists could not truthfully answer without an occasional "I don't know."

From my observation of the effects of this scheme of class work in the Woods Hole course, I believe the following aspects could be carried over with profit into the usual winter zoölogical courses.

1. The introduction of more work with living animals, particularly in the introductory course, and the elimination of much

of the work on formalin specimens. This has been done in the Michigan course as outlined by Shull and his associates, and I believe that the method is applicable in the majority of our laboratories. This requires more work in the presentation of a course, since it is easier as well as cheaper to buy, for example, formalin-preserved earthworms than to keep living ones ready for study at the desired time. It necessitates also an acquaintance with the local animals and their peculiarities of structure and habit, when these are not described in the standard textbooks.

2. Aquaria with living animals should be constantly in the laboratories of appropriate classes even though no work is assigned to be done on them. They will interest our better students in spite of themselves, even in the midst of the crowded winter curricula and outside activities. These aquaria may well include a balanced salt water one.[1]

3. Wherever possible, field work should be included with the work in the first-year course, since it is impossible to understand even the morphology of an animal when seen only in the pickle jar and laboratory environment. Students should not be taken out *en masse*, but in carefully divided groups and the trip should yield definite information which the student will recognize as being an integral part of the course. One section of the year's work in the elementary course, preferably the spring, may well be devoted to field studies which should serve to emphasize many of the problems discussed earlier in the year.

4. A number of approximately equally well-trained instructors should be associated in giving the larger courses. I regard the results from the staff here described as definite proof of the inferiority of the usual method whereby one man, in charge of the course, gives all the lectures and others, poorly trained, often be-

[1] Salt water may be obtained from the sea shore and inexpensively shipped by freight half way across the continent. Living animals for salt water aquaria, together with plants to balance them, may be secured from Mr. G. M. Gray, of the M. B. L. Supply Department. For best results this material should be forwarded soon after definitely cool weather sets in. Mr. Gray always sends more animals than can well be kept and the hardest task is to kill enough at the beginning so that the others may have a fair chance to live. I have kept such an aquarium running for seven months with no trouble other than keeping the water level constant by adding distilled water.

ginning graduate or advanced undergraduate students, give laboratory instruction. I understand the conditions under which this type of instruction has arisen but it is essentially unsound.

The lectures frequently become a routine re-hashing of bygone compilations on phases of the subject of no particular interest to the lecturer, and often quite unrelated to the laboratory work. This is not true in the fortunate laboratories where the lecturer in the large classes is a brilliant master of his subject matter, the English language and the principles of pedagogy, but it holds for too many courses. It is true that a certain coherence of presentation is gained when all of the subject matter passes through one mind, but personal prejudices are likewise transmitted, however unconsciously. Effective teaching is done only when the student is keenly interested and the shifting of lecturers makes naturally for a stimulus of interest.

In the laboratory work, the advantage of the employment of a group of men all well grounded in the essentials of the subject is obvious. The odd findings of an enterprising student can be capitalized, explained on the spot or their source of explanation suggested, when in the ordinary routine of laboratory work such unknowns must of necessity be disregarded by the undertrained or overworked teacher. I am thoroughly convinced that very seldom should instruction be given in college or university by persons under the rank of instructor, with its present connotations, and that courses should be limited or teaching staffs increased until this is possible.

5. The dominant spirit at Woods Hole is research or preparation for research, and this atmosphere does much to color the work of the Invertebrate Course. I am not yet convinced that teaching of undergraduate students stimulates research, or that an instructor who allows himself to be submerged in research should be trusted in an elementary course; but I am certain that, other conditions being equal, the best teaching is done by men actively engaged in some form of research and in a laboratory where the research purpose prevails.

APPENDIX.

In this appendix are given some more technical directions that we have worked out and that are not readily available lesewhere.

I.

Embryology of Rhynchobothrium: Dr. R. H. Bowen.

Take from the spiral valve of the dogfish the ripe, free, milk-white proglottids of *Rhynchobothrium* and place them in finger bowls of fresh sea water; ten or a dozen may be put in one bowl. Care should be taken not to carry over too much of the digestive slime as it is not feasible to change the water once the eggs are laid. If the proglottids are ripe they discharge the eggs at once in a white cloud. The empty proglottids should be removed and the finger bowl set aside without agitating the water. The eggs soon become dark brown or blackish and stick together and to the bottom of the dish in a solid mass. By keeping the eggs thus bunched their concentration can be controlled when removing them for study. Fresh eggs can be examined at once after laying, but they offer nothing of special interest. After not less than three days and up to periods of five to six days, the hexacanth embryos are completed and ready to emerge from the egg cases. Remove a part of the cluster of eggs by scraping them off and sucking up in a pipette and examine under the microscope. The mechanical disturbance of moving them seems to stimulate the embryos, for it is usually easy to find a number of embryos just emerging from the egg-cases; also free swimming embryos and empty egg-cases. Cultures four days old are generally the best.

II.

Approximate Schedule for Stages in Echinoderm Development Under Mid-July Conditions, from the Experience of Miss Christianna Smith.

1. *Asterias forbesi.*

Stages.	Time after Fertilization.
Polar body formation	45–60 minutes.
Two celled	2–3 hours.
Four to eight celled	3–4 hours.
Sixteen celled	4–5 hours.
Late blastula	7–10 hours.
Early gastrula	18–21 hours.
Late gastrula	24–30 hours.

2. *Arbacia punctulata.*

Two celled	1 hr., 30 minutes.
Four celled	2 hrs., 30 minutes.
Eight celled	2 hrs., 45 minutes.
Sixteen celled	3 hrs., 45 minutes.
Sixty-four celled	4 hrs., 15 minutes.
Early blastula	7 hrs., 15 minutes.
Late blastula	14 hours.
Early gastrula	23 hrs., 30 minutes.
Pluteus	Two days.

3. *Echinarachnius parma.*

Two celled	1 hr., 35 minutes.
Four celled	2 hrs., 45 minutes.
Sixteen celled	3 hrs., 15 minutes.
Sixty-four celled	4 hrs., 15 minutes.
Early blastula	6 hrs., 15 minutes.
Late blastula	10 hours.
Early gastrula	21 hours.
Late gastrula	38 hours.
Pluteus	Two days.

III.

Dissection of Amphitrite ornata: Dr. E. F. Adolph.

From a doped or freshly killed specimen obtain some of the cœlomic fluid by making a small slit in the body wall. What is the color and consistency of this fluid? Examine under the microscope and observe the varieties of cells present and their movements. Some of them are reproductive products.

Pin down the specimen, either fresh or preserved, under sea water with the dorsal or bristle-bearing surface upward. With the scissors cut along the mid-dorsal line, piercing only the body wall. Pin the body wall out flat as you cut from head to tail.

Cœlom.—Are there transverse septa or diaphragms? Note the dorsal and ventral mesenteric strands which hold the digestive organs in place. Cut the dorsal ones. How do the details of internal segmental organization correspond to the external organization?

Circulatory System.—This must be observed before further dissection. Find the large, paired, sub-intestinal vessels. Trace them backward as far as you can and forward into the single gas-

tric vessel which continues into the dorsally situated heart. From the heart the branchial vessels go forward to the three pairs of gills. Find the ventral vessel and note its transverse branches, some of which encircle the digestive organs. You may be able to see also the dorsal, neural, lateral and esophageal vessels, all longitudinal.

Digestive System.—Is the mouth ventral or dorsal? It leads into the straight muscular esophagus. How is the latter held in place? Is there a protrusible pharynx? The esophagus opens into the wide straight stomach. Does it show any segmentation? The winding portion of the alimentary tract is the intestine which continues to the anus. Cut open the stomach and note the character of its walls.

Muscles.—Find the transverse-oblique, longitudinal, setal, and circular muscles. Make a rough diagram of the animal through the stomach region, showing the relative positions of the muscles, stomach, and blood vessels.

Nephridia.—Cut through the oblique muscles in anterior somites and expose the nephridia. Quickly sketch the natural position and shape of one of them. How many external openings or nephridiopores do you find? Put a nephridium from a freshly killed worm under the microscope. How do you think the nephridium functions in the living body? The gonadal glands are small white glands ventral to the esophagus. Ventral to them are the larger and yellowish "stomach glands." The nephridia may serve as gonoducts.

Nervous System.—Displace the digestive organs to one side and find the ventral nerve cord. Has it ganglia? Can you find paired transverse branches? What features of the nervous system are metameric? How far does the ventral nerve cord go posteriorly? Anteriorly try to find the sub-esophageal ganglion, the circum-esophageal connectives and the supra-esophageal ganglion. Where would you expect to find sense organs in *Amphitrite?*

Make a list of the special features of the anatomy in *Amphitrite* that are especially significant in view of its habit of living. Compare the anatomy with that of *Nereis.* Which of the two species

do you consider to be the more highly differentiated? Which is better fitted for self-maintainance and self-preservation? Make a table of the functions performed by the body of *Amphitrite* under two headings; those without which the animal or species could not survive, and those which are luxuries. Are any of these functions performed in a way which you consider more efficient by animals you have already studied in this course?

IV.

Injection of Blue Crab (Callinectes sapidus).

The location of incision to reach the heart is shown in Fig. 1. The injecting syringe should be inserted in horizontal position.

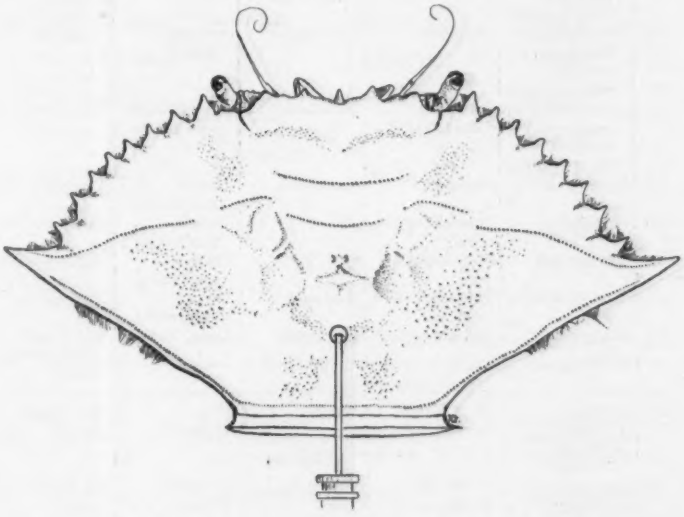

Fig. 1. Dorsal view of the blue crab, showing method of injecting through the heart. Drawn from an injected specimen by Helen Daniels Young.

V.

Table I.

A Comparative Study of Appendages of Common Woods Hole Crustaceans.

Reworked with aid of Mr. J. P. Visscher and Dr. J. A. Dawson from a table made by Dr. G. S. Dodds.

	Lobster.	Blue Crab.	Hermit Crab.	*Hippa.*	*Mysis.*
0	Stalked eye	Stalked eye	Stalked eye	Long stalk	Stalked eye
1	Antennule	Do. small	Do. Short, single	Do. Small, 2 flagella	Antennule
2	Antenna	Do. small	As lobster	Coiled under mouth parts	Antenna large exopod
3	Mandible Palp 3 seg.	As lobster	As lobster	Mandible soft	Mandible small
4	1st Maxilla No endopod	Do. biramous	As lobster	As lobster	1st Max. small
5	2d Maxilla Scaphognathite	As lobster	As lobster	As lobster	2d Maxilla Biram. plumose
6	1st maxilliped biramous, endopod with 2 joints	As lobster	As lobster endopod unjointed	1st maxilliped	Biramous leg
7	2d maxilliped biramous, endopod with 4 joints	As lobster	As lobster	As lobster folded	Biramous leg
8	3d maxilliped biramous, endopod with 5 joints	As lobster flattened	As lobster	3d maxilliped operculate	Biramous leg
9	Large chela	As lobster	As lobster	Digger uniramous	Biramous leg
10	Chelate leg	Non-chelate leg	Do.	Digger uniramous	Biramous leg
11	Chelate leg	Non-chelate leg	Do.	Digger uniramous	Biramous leg
12	Non-chelate leg	Non-chelate leg	Reduced	Digger uniramous	Smaller leg
13	Non-chelate leg	Swimming leg	Leg small	Long, folded	Brood sac from base in female
14	♂ Small modif. ♀ Biram. small	♂ Modified ♀ Biramous	o or left only	♂ o ♀ Small	Swimmeret small
15	Swimmeret ♂ Slightly modif.	♂ Modified ♀ Biram.	Small, left only	♂ Uniram. ♀ Small	Swimmeret small
16	Swimmeret	♂ o ♀ Biram.	Small, left only	♂ o ♀ Small	Swimmeret small
17	Swimmeret	♂ o ♀ Biramous	Small, left only	o	Swimmeret
18	Swimmeret	o	o	o	Swimmeret
19	Uropod Biramous	o	Uropod hooks, left larger	Biramous	Uropod long Lithocyst
20	Telson large	Telson small, pointed	Telson hooked	Do. long, pointed	Do. long, flat

All these have fused cephalothorax.

[1] Tables I. and II. are inserted, although not prepared for publication, because so many teachers of experience have spent time in copying them when given the opportunity.

	Talorchestia.	*Caprella.*	*Oniscus.*	*Argulus.*
0	Non-stalked eyes	Do.	Do.	3 non-stalked eyes
1	Uniramous, short 1st antenna	Do. long	Rudimentary	Minute, 4 segments
2	Uniramous, 2d antenna. ♂ long, ♀ short	Shorter	Well developed	Small
3	Mandible, chitinous teeth and palp	Minute	Mandible	3 and 4 fused in proboscis to form piercing mouth parts
4	1st Maxilla biram. small	Minute	1st Maxilla	
5	2d Maxilla biram. small	Minute	2d Maxilla	Slender
6	1st Maxilliped fused, 2 lobed lower lip	Small	Maxilliped	0
7	Small uniramous hooked	Sub-chelate	1st leg	Suckers
8	Sub-chelate, large in male	Do.	2d leg	Leg-like
9	Uniramous leg	Gill, Brood pouch in female	3d leg	Large leg
10	Uniramous leg	Gill, Brood pouch in female	4th leg	Large leg
11	Uniramous leg pointing backwards	Leg	5th leg	Large leg
12	Uniramous leg pointing backwards	Leg	6th leg	Large leg
13	Uniramous leg pointing backwards	Leg	7th leg	0
14	Swimmeret	Abd. rudimentary	Slender endop. gill exopod, operculum	Unsegmented
15	Swimmeret	Abd. rudimentary	Slender	Unsegmented
16	Swimmeret	Abd. rudimentary	Slender	Unsegmented
17	Jumper large biramous	Abd. rudimentary	Slender	Unsegmented
18	Jumper large biramous	Abd. rudimentary	Slender	Unsegmented
19	Jumper large biramous	Abd. rudimentary	Biramous feelers	Unsegmented
20	———	———	———	———
	Fusion			
	1st thoracic fused to head	1st and 2d thoracic fused to head	1st thoracic fused to head	3 free thoracic segments

TABLE II.

COMPARATIVE MORPHOLOGY AND PHYSIOLOGY OF DIFFERENT GROUPS OF CRUSTACEA. Compiled by Dr. Raymond Binford.

	BRANCHIOPODA	OSTRACODA	COPEPODA	CIRRIPEDIA	MALACOSTRACA
Body Segments	Vary, 13-42. Abdomen no appendages. Apus 50 " " on 17 segments.	Indistinct. No limbs on posterior part.	16. Antennules, antennae mandibles, 2 maxillae, 1 maxilliped=6. 5 with biramous app. 5 abdominal	Indistinct. No abdomen	19. 8 thoracic. 6 abdominal.
Carapace	None, shield or bivalve, attached to only a few segments in front.	Bivalve.	Absent or reduced.	Mantle of several plates Bivalve-shell in larva	Variable. Covers cephalothorax in many.
Tail	Usually a caudal furca	Caudal furca.	Caudal furca.	Caudal furca in most forms.	Telson. Caudal furca in very few.
Eyes	Paired, compound. Nauplius eye usually present.	Compound in a few-Myodocopa. Nauplius-eye present in most.	Nauplius-eye No compound eye.	Nauplius-eye on dorsal surface of stomach	Compound on movable stalks.
Antennules	Small, unsegmented, sensory. Clasping organs in some ♂-Cladocera.	Large. Sensory or locomotory. 8 segments. Some less.	Uniramous. 25 segments with exceptions. Swimming or clasping organs	Used for attachment.	Bears 2 flagella.
Antennae	Anostraca claspers. Notostraca absent or vestigial. Concostraca, Cladocera swimming organs.	Large. Chiefly locomotory. Bears spinning gland in Cytheridae.	Usually biramous, swimming organs.	Disappear in adult.	Scale for exopodite in most.
Mandibles	No palp.	Palp large. Labrum. Clasping organ in Sarsiella.	Sometimes keeps biramous structure in adult	Palp rudimentary.	Lacinia mobilis often present.
Maxillae	1st small, lobed. 2nd mostly small. In Cladocera absent	Absent or variously modified.	Present. 2nd sometimes clinging organs	1st present. 2nd united in lower lip.	Both maxillae present
Appendages	Foliaceous. Respiratory bract.	4 or less back of mandibles. Leaf-like or leg-like. Posterior leg-like.	1 uniramous maxilliped Clinging in some. 5 pairs biramous legs.	6 biramous pairs.	Thoracic & abdominal present & distinct
Locomotion	Swimming by foliaceous appendages. Conchostraca, Cladocera by antennae.	Antennae. A few by antennules.	Antennules, antennae & thoracic appendages	Adults sessile.	Flexible abdomen, tail fin walking legs, swimmerettes
Capturing Food	By gnathobases of appendages.	Large mandibles and labrum.	Mandibles. 2nd maxillae.	Thoracic appendages.	Prehensile legs, several mouth parts.
Excretion	Maxillary gland. Larvae have antennal gland.	Glands at bases of ambulatory legs in some. Cypris antennal & maxill glands	Maxillary gland.	Maxillary glands.	Antennal glands more common.
Reproduction	Sexes separate. No spermatophores. Most sperms spherical. Development in uterus or brood pouch. Nauplius.	Sperms filiform. Some spherical. No spermatophores. Eggs develop in shell, on plants or free in water. Modified nauplius	Spermatophores. Eggs in packets attached at opening of oviduct. Nauplius.	Hermaphrodite. Sperms filiform. Development in mantle cavity. Nauplius with few exceptions.	♀ apertures 6th, ♂ on 8th thoracic segment. Spermatophores.

VII.

Laboratory Work on Limulus: Dr. R. H. Bowen, Dr. C. L. Parmenter and Mr. D. B. Young.

In addition to the work outlined in Drew's manual, the following studies can be readily and satisfactorily made:

I. *Method of Feeding.*—Place a large *Limulus* on its back and wait until it is perfectly quiet. Then place bits of mussel, drops of fluid from a macerated mussel, and bits of *Nereis* on the spiny bases of the legs and note the rôle played by each appendage.

II. *Dissection.*—A longitudinal section of *Limulus* cut just a little to one side of the median plane shows that the various organ systems are arranged in layers. Suspended from the dorsal carapace is the tubular heart surrounded by the pericardium. Below the heart is the digestive tract. This consists of the esophagus, a tube which leads forward to the stomach or proventriculus, which turns upward and backward and opens into the straight intestine. At the junction of the esophagus and proventriculus is a plate of skeletal material, the endocranium, which continues back through the cephalothorax. Below this internal support is the central nervous system. Such a dissection, although useful, does not show the various organs as well as does a dorsal view. Therefore the following method is advised. Students will work in pairs and trace out in succession the vascular, the digestive, and the nervous systems.

1. For dissection use adult, live animals not over 10 to 12 inches across the carapace. The larger ones have a very strong exoskeleton to cut through. Place the animal on its back. Cut off the legs leaving the coxal joint attached to the body. Cut around the carapace as shown by the dotted line in Fig. 2. Use a heavy knife, such as a well-worn oyster knife. Begin at the point marked "1" and keep well in from the edge to avoid the strong trabeculæ which pass dorso-ventrally along the border. Then go back to the abdomen and make the cuts marked "2" and "3." After this cut through the interarticular membrane on the dorsal side of the caudal spine.

The spine and ventral piece of exoskeleton may then be lifted up from the dorsal carapace and the cuts "2" and "3" carried

forward allowing the knife to follow the path of least resistance. Do not cut too far from the median line on account of the trabeculæ. Finally separate the ventral leaf with the viscera attached from the dorsal leaf of the exoskeleton which may be thrown away. Use the hand to make the separation. Cut the muscles

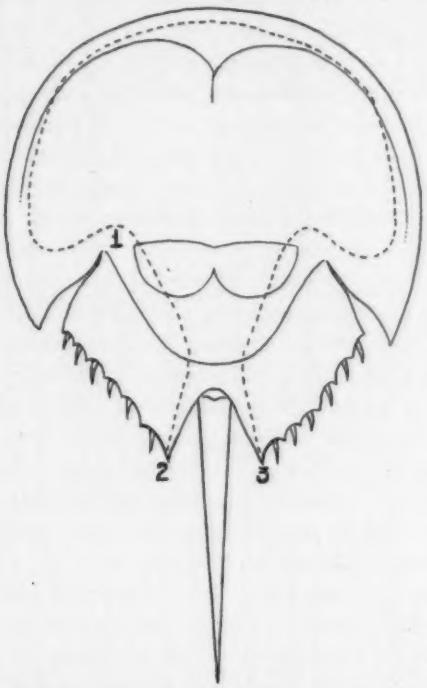

FIG. 2. Diagram of ventral view of *Limulus*, showing location of line of primary incision.

where necessary with a scalpel but take due care not to injure the pericardium and heart lying along the mid-line. Finally lift out the whole of the visceral mass and place, dorsal side up, in a deep crystallization dish. Continue the dissection under sea water.

2. The mush which covers the lateral parts of the animal is composed of gonad (orange) and liver (yellowish-green).

3. The heart[1] and its vessels are more or less exposed when the carapace is removed. Carefully free the pericardial area from gonad, etc., preserving if possible the frontal artery, the aortic arches, and the lateral arteries. In some cases the collateral arteries and their fusion to form the superior abdominal artery can be seen. Some of the branchio-cardia canals, which return blood from the gills to the pericardium, can also be identified. Note also the median cardiac nerve and make out as much as possible of the heart itself and its eight pairs of ostia. Then have your dissection demonstrated by an instructor. (The vascular system is not well shown in such fresh material, but the main features can usually be indicated.)

4. Remove the heart exposing the digestive system. Clear up the neighborhood in the same way as for the vascular system. Take care to preserve the large ducts by which the liver communicates with the intestine. The digestive system is a very simple one and will require little or no demonstration by the instructors.

5. Cut the digestive tract near the posterior end and reflect it forward. This exposes the cartilaginous endocranium. The central nervous system lies directly beneath the endocranium, which must first be removed in order to expose the nerve ring. This can best be done by cutting along the median line of the cartilage with scissors, beginning at the anterior end and taking great care not to damage the tissues beneath. Reflect the halves of the endocranium laterally. This exposes the nerve ring and ventral nerve cord. Carefully clear up the area; work the nerves free with fine curved forceps or needles. Take great care to damage the nervous structures themselves as little as possible. Finally work back along the ventral nerve cord and expose the five ganglia; do not lift the cord as the nerves radiating from the ventral surface of the ganglion will thus be completely destroyed. Special

[1] The circulatory system of young specimens (carapace of three inches) may be injected through the heart by inserting the needle in the median line in the groove between the carapace and abdomen. The entire circulatory system may be followed by removing the dorsal part of the carapace, since the injected vessels may be readily seen through the semi-transparent ventral body wall. Larger specimens may be similarly injected with some advantage in dissecting.

care should be taken in clearing up the area just anterior to the forebrain. Otherwise the delicate olfactory nerves and the nerves to the eyes will be entirely destroyed. Similar care should be taken to preserve the long and delicate nerves which pass from the posterior border of the main nerve ring to the chilaria and operculum.

The nerves arise from the central nervous system in groups of two; one dorsal, called the hæmal, and one ventral, the neural From the nerve ring, eight pairs originate; six large anterior ones and two smaller posterior ones. In addition a median and two lateral olfactory nerves; a median and two lateral eye nerves, arise from the front of the ring. Great care must be used in finding them. The ventral nerve cord has five ganglia with pairs of hæmal and neural nerves from each. The last three sets of nerves join the posterior end of the cord. Find as many of the nerves as you can. An instructor will demonstrate and complete the dissection where possible.

6. Study the arrangement of the coxal glands, the brick-red tubes which lie lateral to the nerve ring. This is the excretory system.

In the absence of an instructor the following papers will be of assistance:

Lankester, E. Ray. Is *Limulus* an Arachnid? Quart. Journ. Micr. Sci., 21, N. S., 1881.

Placed *Limulus* with the Arachnida.

Patten and Redenbaugh. Studies on *Limulus*, I. The Endocranium of *Limulus, Apus* and *Mygale*. Jour. Morph., 16, 1899. Studies on *Limulus*, II. The Nervous System of *Limulus* with Observations upon the General Anatomy. Jour. Morph., 16, 1899.

Morphological account; good figures; direct aid in dissection.

Patten and Hazen. Development of the Coxal Glands, Branchial Cartilage and Genital Ducts of *Limulus polyphemus*. Jour. Morph., 16, 1900.

Patten, Wm. The Evolution of Vertebrates and their Kin. Blakiston, Phila., 1912.

Complete bibliography.

VII.

TABLE III.

CLASS LIST AS POSTED ON THE AFTERNOON OF AUGUST 8, 1920, ON RETURNING FROM A COLLECTING TRIP TO THE NORTH FALMOUTH FLATS.

(Collecting from 3:20 to 6:00; low tide 5:00.)

PORIFERA
Cliona
Microcione
Tethya

CŒLENTERATA
Edwardsia
Eloactis
Hydractinia
Pennaria
Podocoryne
Obelia commissuralis
Sagartia leucolena
luciæ
modesta
Sertularia
Eudendrium album

PLATYHELMINTHES
Cerebratulus
Lineus
Micrura
Stylochus
Syncœlidium

NEMATHELMINTHES
Pontonema

ECHINODERMA
Autolytus
Arabella
Arenicola
Cirratulus
Clymenella
Diopatra
Enoplobranchus
Glycera sp.
Harmothoe
Hydroides
Lepidonotus
Lumbrineres tenuis
Nereis limbata
virens
Pectinaria
Phascolosoma
Phyllodoce
Pista
Platynereis
Podarke
Polycirrus
Sabella
Sabellaria
Scoloplos acutus
fragilis
robustus
Spirorbis
Sthenelais
Maldane

BRYOZOA
Ætea
Bugula
Crisia
Flustrella
Lepralia
Membranipora
Schizoporella

ARTHROPODA
Amphithoe
Balanus
Callinectes
Caprella
Carcinides
Chiridotea
Crangon
Gammarus
Heteromysis
Idothea baltica
metallica
Libinia dubia
emarginata
Limulus
Pagurus pollicaris
longicarpus
Palæmonetes
Panopeus
Pinnixa
Orchestia
Talorchestia
Ovalipes
Uca
Virbius
Hausterius
Jæra
Ericksonella

MOLLUSCA
Chætopleura

PELECYPODA
Anomia
Cumingia
Ensis
Lævicardium
Macoma
Mactra
Modiolus demissus
modiolus
Mytilus
Nucula
Pecten
Petricola
Solemya
Tellina
Teredo
Venus
Mya
Ostræa

GASTEROPODA
Acmea
Bittium
Busycon
Cotumbella avara
lunata
Crepidula convexa
fornicata
plana
Lacuna
Littorina litorea
palliata
rudis
Nassa obsoleta
trivittata
Natica duplicata
heros
Odostomia
Urosalpinx

CHORDATA
Amaræcium
Botryllus
Dolichoglossus
Molgula
Perophora
Styela

126 species

CILIARY AND MUSCULAR LOCOMOTION IN THE GASTROPOD GENUS POLINICES.

MANTON COPELAND,

SEARLES BIOLOGICAL LABORATORY, BOWDOIN COLLEGE.

INTRODUCTION.

Although gastropod locomotion has often been made a subject of investigation by naturalists and physiologists with the result that the characteristic muscular form of progression is now fairly well understood, it appears that little is known concerning the peculiar and equally interesting activities of cilia which occur on the feet of numerous species of snails, and the part they may play in locomotion, notwithstanding the fact that Quatrefages as early as 1843 believed them to be locomotor in function. A study of pedal cilia in two species of marine gastropods belonging to the genus *Alectrion* led me to conclude not only that locomotion was due to ciliary action, but also that this action, unlike that of ordinary cilia, was either directly or indirectly under the control of the nervous system (Copeland, '19). Recently similar studies on the genus *Polinices* have been carried on at the Marine Biological Laboratory at Woods Hole, the results of which are here recorded. This group appears to contain the largest animals which have been reported as exhibiting ciliary locomotion. The snails also have a muscular form of progression, thus showing a combination of locomotor methods which I believe has not been described for marine gastropods.

Most of the work has been done on *Polinices* (*Neverita*) *duplicata* (Say), although the more northern species *Polinices* (*Euspira*) *heros* (Say), which is closely similar to the preceding, has been studied to some extent. Unless otherwise specified, observations here noted refer to the former species.

The large foot of *Polinices* is differentiated anteriorly into a well-developed propodium which is prolonged over the head region and a part of the shell. This organ, bluntly pointed at the anterior end, is capable of great extension and freedom of movement, and when protruded bears a striking resemblance to the mammalian

tongue. The remaining portion of the foot is elliptical in outline and possesses a dorsal area which is reflected over a section of the shell. The ventral pedal surface is covered by ciliated epithelium which is richly supplied with mucus. The dorsal surface of the propodium is also ciliated. The effective strokes of the cilia are directed toward the posterior end of the foot and there is no indication that they are ever reversed.

Ciliary Behavior and Locomotion.

When *Polinices* is placed in a glass dish filled with sea water, it usually exhibits a gliding type of locomotion quite distinct from another form of progression which it sometimes employs involving marked muscular contractions of the foot. The former method may be considered first. A close inspection of the foot as it slides over the substrate fails to reveal any indication of rhythmic pedal waves, all regions of the organ moving at the same speed over a thick layer of mucus. Slight muscular contractions produce a rippling motion along the anterior border of the propodium. The same mode of progression is seen when the snail is moving over a sandy or pebbly substrate, and is essentially like that described for *Alectrion obsoleta* (Parker, '11; Copeland, '19) and *Alectrion trivittata* (Copeland, '19), where locomotion has been shown to be due to cilia beating in mucus which covers the sole of the foot and adheres to the substrate (Copeland, '19). A young *Polinices heros* was also noted moving in a similar manner in an inverted position along a band of mucus laid down on the surface film of the water.

In *Alectrion* it was found that actively beating cilia are characteristic of a moving snail, whereas they are quiescent, or nearly so, when the animal is motionless, as would be expected if the cilia function as locomotor organs. Accordingly *Polinices* was first studied with the object of determining whether the behavior of the pedal cilia was like that recorded for *Alectrion*. An adult snail is too large for successful microscopic examination, but a young individual (*P. heros*) whose foot measured 22 mm. in length was observed while moving over a slide in a small amount of water and the cilia on the thin posterior portion of the foot could be seen beating actively. The same animal was again examined when resting with a partly contracted foot in a culture slide, and at this time

all the cilia discernible were either quiescent or showed but slight activity over local areas. The behavior of the pedal cilia of an adult *Polinices* is unquestionably like that of the immature, for when the progression of one is suddenly prevented by holding back the shell, the mucus on the bottom of the foot is driven posteriorly by the beating cilia and objects such as sand grain, pieces of filter paper or even a pin are swept along with it. If now the snail is removed from the water, the propodium may be extended and moved about, at which time the mucus glides over the entire ventral pedal surface and the flicker of beating cilia can be plainly seen by the use of a lens. On the other hand, if the animal is quiescent, either in the water or when held out of it, there is no movement of mucus and in the latter position no indication of ciliary activity is distinguishable.

Polinices placed upside down in a dish of sea water shows a righting reaction which is characteristic. The tongue-like propodium is extended, and the tip directed downward and backward until the ventral anterior margin of the foot is applied to the glass. The attached surface of the propodium is then advanced until the inverted shell lies over the right anterior border of the foot. If the animal does not succeed in applying more of the surface of the foot to the glass, the shell and posterior part of the foot move backward as the propodium progresses, the whole body tending to circle clockwise. If during this activity sand grains are sprinkled over the foot, they are carried posteriorly with the mucus by the beating cilia. No muscular waves are to be seen on the portion of the propodium which is adherent to the glass and locomotion at this time is evidently accomplished by the cilia. On failing to right itself the snail rests with the propodium still in contact with the substrate, or more frequently with the organ withdrawn and held in a somewhat contracted condition with its ventral surface upward. Sand grains now remain on the foot where they are placed and there is no movement of mucus. When, however, the righting activity is resumed the sand and mucus again move down the foot. The righting of the body is finally accomplished after more of the pedal surface is applied to the substrate, particularly that portion along the right side posterior to the propodium, when the rest of the foot and the shell are thrown over to the left. The expanded

hind part of the foot shows no muscular movement during the entire reaction.

The preceding observations lead to the conclusion that pedal cilia are beating actively at the time of locomotion, when the foot is functioning as a righting organ, and when it is being moved about as the snail is held out of the water, and that cilia are quiescent, or nearly so, when the animal is at rest either in the water or out of it. The results of certain experiments may now be recorded which in my opinion leave little doubt that these cilia function as organs of progression.

A comparison of the rate of locomotion with the speed at which objects were driven over the pedal surface by beating cilia, or what may be termed the ciliary rate, was obtained in the following way. A snail which was moving around the periphery of a shallow glass dish filled with sea water was stopped by holding back the shell, but grains of sand adhering to the mucus were carried along the under surface of the foot by the active cilia as the animal attempted to continue its course. By the use of a millimeter rule and mirror placed beneath the dish it was possible with a stop-watch to obtain records of the rate of their movement. One of the lateral margins of the foot was slightly curled upward and some of the records were taken as the sand passed over this area; other grains were timed as they moved with the mucus nearer the central and posterior portions of the expanded foot. The average rate of movement over a distance of 5 mm. in ten trials was found to be 2.7 seconds. Immediately after the ciliary rate was obtained the snail was allowed to proceed over the bottom of the dish and ten records of its speed were secured, which showed that it was moving at the average rate of 5 mm. in 2.6 seconds. The remarkably close correspondence between the locomotor and ciliary rates strongly supports the view that progression is accomplished by ciliary action.

Watching *Polinices* glide over the bottom of an aquarium one gains the impression that the animal is too heavy to be propelled merely by the lashing of cilia in a film of mucus spread over the substrate. That such a conclusion is in reality a false one was proved by a few simple tests. As has been pointed out, the pedal cilia are active when the snail is attempting to right itself, and at that time it is possible to determine their driving power by placing

objects on the expanded ciliary surface. In this manner it was found that a five-cent piece and good-sized pebbles were readily moved by the beating cilia. Following these preliminary tests, a carrier weighing slightly over half a gram was made by fastening a glass ring on a 22 mm. cover glass. A 20-gram weight was then set on the ring, and the cover glass placed on the upturned middle portion of the foot, which in turn was supported so that the cilia were at about the level of the surface of the water. When the snail began the righting reaction the beating cilia moved the mucus and carrier with its load toward the posterior end of the foot. One of the animals tested in this way weighed between 8 and 9 grams in water and had a pedal ciliated surface with foot expanded of about 54 square cm. Thus between 4 and 5 square cm. of active cilia were able to move not less than 20 grams, whereas during locomotion the task of moving 8 grams was distributed over an area of 50 square cm. The largest specimen of *Polinices duplicata* which I was able to obtain weighed only 36 grams in water and its locomotor surface measured about 132 square cm. Although the resistance of the water to be overcome by the moving animal is a factor to be considered in any detailed calculation of the work required of the locomotor apparatus, the results of these tests leave no doubt as to its adequacy.

Although *Polinices* was once observed to move a short distance clinging to the under side of a glass plate with the propodium unattached to the glass, it is the anterior border of this organ which as a rule holds most tenaciously to the substrate when one attempts to dislodge the snail. Several times I have seen one move up the glass side of an aquarium until it came to the surface of the water, when it turned the propodium downward, or perhaps parallel to the surface, and lifted it from the glass. Soon after relinquishing this anterior hold it slipped to the bottom. Moreover, it is able to cling to the under side of a glass plate by the propodium alone and to advance several centimeters by muscular action. Evidently, therefore, this specialized portion of the foot has considerable power of suction, although adhesion by means of mucus is in all probability the principal mode of attachment for the foot as a whole. When the snail is moving the anterior margin of the propodium is in close contact with the substrate and exhibits con-

tinuous though slight muscular contractions. It was conceivable, therefore, that it played some important part in effecting the locomotion of the animal which had been overlooked. In order to test this possibility the anterior portion of a snail's foot was removed so that the locomotor function became restricted to the mid and hind regions. Less than three days after the operation the snail was moving about the aquarium so much like other individuals that it could not be distinguished from them without examining its foot. When moving rapidly, ten records of its speed were taken which showed an average locomotor rate of 5 mm. in 3 seconds. Five records obtained at other times when it was most active averaged 2.6 seconds for the same distance. Another, normal animal, whose speed of locomotion was noted before the preceding tests were made, was moving at the rate of 5 mm. in 3.2 seconds (average of ten trials). After completing this experiment more of the anterior region of the foot was cut away so that there was nothing left which came in contact with the substrate which could possibly be regarded as a portion of the propodium. Again locomotion was not interfered with, and the snail glided both slowly and rapidly over the bottom of the aquarium. It is clear, therefore, that the propodium is not a part of the pedal mechanism which is essential for locomotion.

Muscular Locomotion.

In sharp contrast to the ciliary type of locomotion in *Polinices* is one involving the formation of rhythmic pedal waves. This is a muscular form of progression which begins by the posterior end of the foot being drawn forward initiating a dark transverse band or wave, which travels over the ventral surface of the foot until it reaches the anterior portion when the propodium is thrust ahead. At about the time the propodium is fully extended the hind end of the foot is again contracted and a second wave is produced. This muscular action causes a slight humping of the body, so that the shell rises after the posterior contraction and falls again as the wave passes forward. If the animal is moving very rapidly the anterior end of the foot may be lifted well above the substrate when the wave reaches it, extended and brought down again with considerable force. Although the wave may not be apparent along

the lateral margins of the foot, it does at times extend to this region when, under favorable conditions for observation, it is perfectly clear that the wave is a band-like area of the foot which is temporarily raised above the substrate and moved forward. This interpretation of the pedal wave was offered by Parker in 1911, and later (1917) was shown by him to be true in the case of *Aplysia*. Olmsted ('17) also came to the same conclusion from his studies of Bermudian gastropods.

The speed of muscular locomotion was compared with that dependent on ciliary action alone. A snail whose foot measured about 10.5 cm. in length by 5.7 cm. in width was moving over the bottom of a lead-lined tank in which the water was over 3 cm. deep. By taking five records and averaging them, it was determined that five complete waves passed over the foot in 27.6 seconds, during which time the snail moved forward 7.8 cm. Thus a wave appeared on the average every 5.5 seconds and advanced the foot approximately 1.5 cm. It was moving, therefore, at the rate of one centimeter in about 3.5 seconds. A larger individual with a foot about 15.5 cm. long and 10.7 cm. wide moved much faster. When the results of five trials were averaged it was found that the snail was travelling at the rate of 18.5 cm. in 31.6 seconds, or stated as in the preceding case, a wave started every 6.3 seconds and moved the foot ahead about 3.7 cm. Accordingly the rate of locomotion was approximately a centimeter in 1.7 seconds. Comparing these locomotor rates with those determined for several snails moving without rhythmic contractions, it is evident that the muscular form of progression is faster. For example, the smaller, more slowly moving individual of the two noted above was timed as it glided by ciliary action over the glass bottom of a dish of sea water, a favorable substrate for this type of movement. Twenty records of its speed over distances of one centimeter were taken. The fastest noted was 5 seconds, whereas the series averaged 5.7 seconds.

Undoubtedly pedal cilia are beating when waves are passing over the foot, for they unquestionably are when the snail is burrowing into the sand and the same sort of contraction is in progress. The muscular form of locomotion may pass into the purely ciliary one by the gradual reduction of rhythmic pedal movements

until they entirely disappear. Ciliary progression was observed more frequently than muscular in the case of snails confined in aquaria. The latter type now and then appeared when the animals were moving in a lead-lined tank, over a glass surface and often when they were climbing up the sides of glass aquaria. Ciliary locomotion was successfully accomplished over pebbly bottoms as well as sandy ones. In the former situation the foot, especially the propodium, molded its surface to the irregularities of the substrate and appeared as if it were flowing over them. The remarkable effectiveness of this form of progression is readily understood, however, if it is remembered that irrespective of the character of the substrate the cilia are always beating against a thick layer of mucus which adheres to the underlying materials, and forms a smooth bed over which the foot can readily glide.

If, when moving over a pebbly bottom, the propodium is thrust beneath the stones, the foot is worked forward by muscular contractions, the anterior end in contact with the glass bottom of the aquarium. Thus when considerable resistance is to be overcome the muscular form of progression is employed. This fact is well illustrated by the behavior of a snail burrowing into the sand. Rhythmic contractions go on as in ordinary locomotion. The propodium is directed downward, and when the pedal wave reaches the organ it is extended and worked farther forward and downward into the sand. By holding back a burrowing animal, sand grains may be seen travelling along the sole of the foot and also over the dorsal surface of the propodium, showing that the cilia are beating. By this procedure a snail may completely disappear beneath the sand in less than a minute and a half to a little over two minutes. When the anterior end of the foot was cut off, *Polinices* could not burrow successfully. By continued pedal contractions it was able to work into the sand somewhat, but at the end of about an hour it rested with a part of the shell still exposed. The propodium, therefore, although not necessary in locomotion, is essential for burrowing.

Ciliary Control.

It is now well established that the transmission which excites activity over ciliated epithelia is not ordinarily directed through

nerves, but through the protoplasm of the ciliated cells, a kind of transmission which on account of its resemblance to the nervous type has been designated neuroid. In the course of investigations on the behavior of cilia on the excised foot of the snail *Alectrion trivittata* (Say) the wave-like spread of action was clearly observed (Copeland, '19). Cilia quiescent over a limited area of the foot suddenly became active, others close by then began beating, and as the wave of action continued many were in motion. After a time they came to rest again. To account for this behavior, and the fact that cilia start beating over the entire ventral surface of the foot when the snail begins locomotion, one might assume that resting cilia are excited to movement in some definite region of the foot through impulses received from the nervous system, and that neuroid, or intraepithelial transmission, spreading from this center, brings about the activity of the remaining cilia. This possibility is rendered improbable, however, from the fact that if two observers are watching carefully the sand-covered surface of the foot of *Polinices* when the snail begins the righting reaction, no difference can be detected in the time when the sand grains begin movement, although the two points under observation may be ten centimeters apart.

In order to gain more definite information on the method of transmission in *Polinices* the following experiments were tried. After anesthetizing a snail in magnesium sulphate, the epithelium near the center of the foot was cut in such a way that an area 25 mm. long and 18 mm. wide was isolated from the surrounding epithelium. When the animal became active again after the operation the cilia on the isolated epithelium behaved the same as those over other parts of the foot, as far as could be determined by testing with sand grains. As long as the snail rested upside down there was no movement of the mucus or sand on the foot, but when it attempted to right itself ciliary activity began on the isolated patch of epithelium, as well as elsewhere, and continued until the animal rested again. Following this test more of the epithelium was cut away so that the subepithelial tissues were exposed in a well-defined band completely surrounding the epithelial island. The ciliary behavior was the same as before. The test was repeated on *Polinices heros,* an area of epithelium 13 to 15 mm. in

diameter being separated from the surrounding portions near the center of the foot. Again the ciliary action over the isolated part was coördinated with that of the rest of the pedal surface. These experiments prove that impulses exciting ciliary action may be transmitted elsewhere than through epithelial cells, and point to a subepithelial or nervous pathway. Other tests on two examples of *Polinices heros* substantiated this view. The tissue lying beneath the epithelium was severed in a plane parallel with the surface. The cuts extended inward from near the border of the foot posterior to the propodium, leaving most of the overlying epithelium in contact with that of the rest of the foot, but isolating it from the deeper portions of the organ over an area, in one case, about 15 mm. long by 4 to 7 mm. wide, and in the second instance, approximately 8 mm. by 13 mm. Many tests were made during righting reactions, and although sand grains moved with the mucus directly in front and back of the areas cut under, and also to one side of them, they remained quiescent over the isolated regions themselves. The operation caused the epithelium to become somewhat contracted, but numerous tests on normal individuals have shown that a wrinkling of the pedal surface does not in any mechanical way inhibit the beating of cilia.

Conclusions.

The view that gastropod locomotion is not always a muscular process is well supported by the behavior of *Polinices* where two types of progression occur in a single species, one of which is immediately recognized as muscular, whereas the other presents entirely different features and upon analysis appears to be dependent on ciliary action.

The general ciliary behavior in *Polinices*, and especially the results of the experiments just described, uphold strongly the conclusions already drawn from a study of two species of the genus *Alectrion*, namely, that the control of ciliary action has been taken over by the nervous system, perhaps directly, or conceivably through connections with muscle or mucous cells associated with the ciliated cells. Intraepithelial transmission may play some part in effecting the spread of ciliary action, but evidently impulses

directed over deeper pathways are the primary ones concerned in regulating this movement.

LITERATURE CITED.

Copeland, M.

'19 Locomotion in Two Species of the Gastropod Genus *Alectrion*, with Observations on the Behavior of Pedal Cilia. BIOL. BULL., Vol. 37, pp. 126–138.

Olmsted, J. M. D.

'17 Notes on the Locomotion of Certain Bermudian Mollusks. Jour. Exp. Zoöl., Vol. 24, pp. 223–236.

Parker, G. H.

'11 The Mechanism of Locomotion in Gastropods. Jour. Morph., Vol. 22, pp. 155–170.

Parker, G. H.

'17 The Pedal Locomotion of the Sea-Hare *Aplysia californica*. Jour. Exp. Zoöl., Vol. 24, pp. 139–145.

de Quatrefages, A.

'43 Mémoire sur l'Éolidine paradoxale (*Eolidina paradoxum* Nob.). Ann. Sciences Naturelles, Sér. 2, Tome 19, pp. 274–312.

THE EFFECT OF IODINE AND IODOTHYRIN ON THE LARVÆ OF SALAMANDERS. III. THE RÔLE OF THE IODINE IN THE SPECIFIC ACTION OF THE THYROID HORMONE AS TESTED IN THE METAMORPHOSIS OF THE AXOLOTL LARVÆ.*

E. UHLENHUTH.

(Received for publication September 11, 1921.)

The rôle of the iodine in the specific action of the thyroid hormone is still an undecided question. The most recent investigations on mammalia have led to the conclusion that the inorganic iodine as such is incapable of bringing about effects identical with the specific effects of the thyroid hormone.

Kendall[1] has shown that the specific reactivity of the thyroid hormone (thyroxin) as manifested by its capability of abolishing myxedema, mitigating cretinism, and raising metabolism depends chiefly on the presence, in it, of a CO-NH group. Iodine, although it may *increase* the specific reactivity of thyroxin, does not *produce* it, as is shown by the ineffectiveness of derivatives in which merely the hydrogen on the imino group has been replaced by acetyl without changing the position of the iodine atoms. Kendall, therefore, has been forced to conclude that the iodine is not responsible for the specific action of the thyroid hormone.

Quite in line with Kendall's viewpoint are Leo Loeb's recent experiments on the compensatory hypertrophy of the guinea pig's thyroid. Loeb found that the inhibitive effect in this kind of hypertrophy is specific for the thyroid hormone,[2] while inorganic iodine as such is incapable of preventing the compensatory hypertrophy of the thyroid gland. Leo Loeb, therefore, claims, like Kendall, that the effect of iodine is not identical with the effect of the thyroid hormone.

* From the Laboratories of the Rockefeller Institute for Medical Research.

[1] Kendall, E. C., *Endocrinology*, 1917, i, 153–169, and 1919, iii, 156–163. Plummer, H. S., and Boothby, W. M., *Amer. J. Physiol.*, 1921, lv, 295.

[2] Loeb, L., *J. Med. Res.*, 1920, xli, 481–494, and 1920, xlii, 77–89.

As Leo Loeb rightly points out, the only facts seriously in the way of his own and Kendall's viewpoint are the experiments on amphibians, especially those of Swingle,[3] which have shown that administration of inorganic iodine causes precocious metamorphosis of tadpoles. Loeb overcomes this obstacle by assuming that the amphibian metamorphosis is not so much an indicator of thyroid activity as of the action of iodine as such. Kendall[4] likewise sees himself compelled to rule the amphibian metamorphosis out of the phenomena caused by the specific action of the thyroid hormone, and assumes now that thyroxin has a twofold effect, one on myxedema and basal metabolism, which is specific to the thyroid hormone and dependent on the peculiar chemical constitution of it, and one on the amphibian metamorphosis, which is merely due to the iodine contained in the thyroid hormone and not specific for the latter one, but for the inorganic iodine as such.

In a previous article I have shown that the interpretation given by myself and others to Swingle's experiments is not correct.[5] Although it may be true that the administration of inorganic iodine enforces metamorphosis of the tadpoles, it must be kept in mind that from the *effect of the administration of a substance* one can not, with any certainty, conclude upon the *effect of the administered substance*, unless the fate which this substance undergoes in the body is known. As to the fate of the inorganic iodine in the tadpole body, there is much evidence to show that it does not produce metamorphosis in the form of inorganic iodine, but after the thyroid—or in the absence of this gland other tissues similar in their function to the thyroid gland—has elaborated from it the thyroid hormone. The results on iodine-fed tadpoles, therefore, are neither new nor are they surprising as far as the ability of the tadpole thyroid goes to produce more "active thyroid hormone," if more iodine is administered; it is long known that the mammalian thyroid possesses exactly the same ability as shown by Marine and Rogoff in quantitative experiments.[6] The only thing new in Swingle's experiments is the discovery that the thyroid apparatus

[3] Swingle, W. W., *J. Exper. Zoöl.*, 1918–19, xxvii, 397–415.
[4] Kendall, E. C., *Amer. J. Physiol.*, 1919, xlix, 136–137.
[5] Uhlenhuth, E., Endocrinology (in press).
[6] Marine, D., and Rogoff, J. M., *J. Pharm. and Exper. Ther.*, 1916, ix, 1–10.

of the tadpoles or certain parts of it do not possess the ability of storing the thyroid hormone after its elaboration. The tadpole experiments in themselves, however, do not decide anything as to the rôle of the inorganic iodine in the action of the thyroid hormone. When viewed in the light of the experiments on salamanders they support the conception that inorganic iodine as such does not play an essential rôle in the effects characteristic for the thyroid hormone, and that the amphibian metamorphosis can be effected *only* by the thyroid hormone.

I have shown[6] that inorganic iodine, when administered to such amphibians in which the control of the excretion of the thyroid hormone is similar to that in mammalians—*i.e.*, in which no hormone can escape from the thyroid in the absence of the action of a particular releasing mechanism—is completely unable to produce the amphibian metamorphosis. In the experiments to be reported below I have used so-called axolotl larvæ. The axolotls, which are the larvæ of the species *Ambystoma tigrinum*, are characterized by an inhibition of metamorphosis resulting in a considerable prolongation of the larval period. It is possible, therefore, to extend, in this species, the experiments over long periods and to administer excessively large quantities of iodine. The ineffectiveness of such large quantities of inorganic iodine as compared to the prompt effect of amounts of iodothyrin containing only minute quantities of iodine disproves, in a quantitative manner, that inorganic iodine is the active principle of the thyroid hormone in the amphibian metamorphosis.

Experiments.

From about 100 larvæ of the salamander *Ambystoma tigrinum*, which had developed into so-called "axolotl larvæ,"[7] 9 larvæ were selected for the experiment to be reported here. They were kept in ordinary tap water and fed earthworms in the beginning, beef liver later on.

Beginning of Experiment (December 9, 1920).

No. 1 measured 189.0 mm.; No. 2, 160.6 mm.; and No. 3, 194.4 mm. All three animals were completely larval (large gills, large

[7] The axolotl larvæ were collected in the Colorado Rocky Mountains in August and September, 1920.

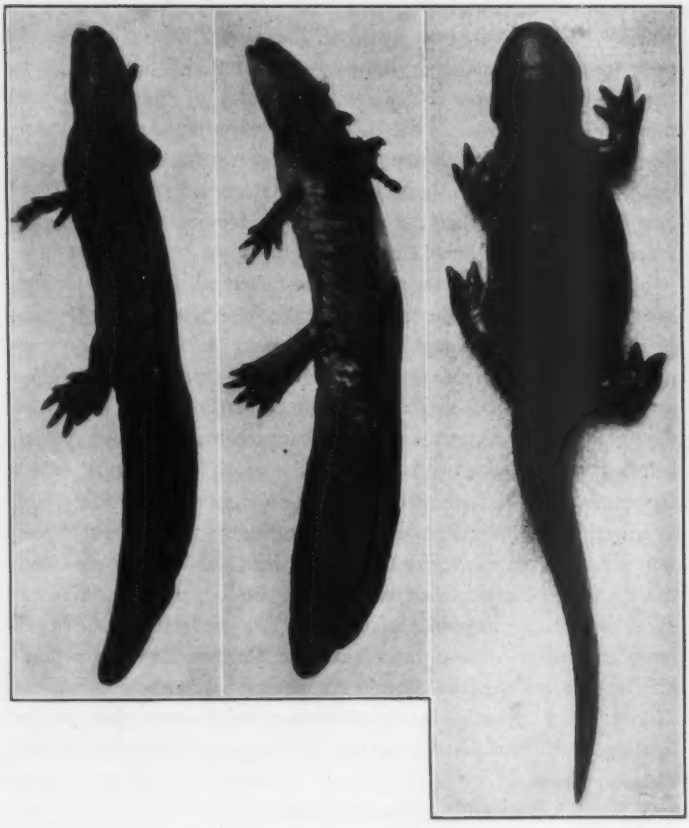

FIG. 1. Control larva 134 days after the beginning of the experiment. Note the shortened gills and the almost complete absence of the anterior portion of the ventral fin.

FIG. 2. Iodine-fed larva 134 days after the beginning of the experiment. Note the long gills and the completeness of the fin. This larva, although large amounts of inorganic iodine were administered to it, is less far advanced towards metamorphosis than the control animal.

FIG. 3. An animal to which iodothyrin was administered, photographed 134 days after the beginning of the experiment. This animal metamorphosed 13 days after the first administration of iodothyrin; it was a completely metamorphosed terrestric salamander when the photograph was made, and had been so several months before it was photographed.

fin on ventral and dorsal side of tail, eyes not bulging out) and No. 3 was neotenous (a sex mature male whose cloacal lips were swollen and warty). These 3 larvæ served as controls.

No. 4 measured 184.9 mm.; No. 5, 158.5 mm.; and No. 6, 214.0 mm. All 3 larvæ were completely larval; No. 6 was a sex mature male. This set of larvæ was used for the iodine experiment.

No. 7 measured 179.8 mm.; No. 8, 154.2 mm.; and No. 9, 184.5 mm. Again each animal was completely larval; the third one, however, presented no external sign of sex maturity (it was probably a female). This set was used for the iodothyrin experiment.

It will be noticed that each larva of each set was comparable to a corresponding larva of the two other sets, as regards size and stage of development. The larvæ selected for the iodothyrin experiment, however, were smaller in average than the larvæ of the two other sets. This selection was made in order to be sure that in case the larvæ kept in iodothyrin should metamorphose before the other larvæ their earlier metamorphosis was not due to a more advanced stage at the beginning of the experiment, but to the specific effect of the iodothyrin.

On December 9, 1920, the larvæ of the second set (Nos. 4, 5, 6) were placed into an iodine solution containing 5 drops of a 1/20 M stock solution of inorganic iodine (in 95 per cent. alcohol) per 1,000 c.c. of water, while the larvæ of the third set (Nos. 7, 8, 9) were put into water containing 0.1 gm. of Bayer's iodothyrin per 1,000 c.c. of water. The larvæ of the first set (Nos. 1, 2, 3) served as controls.

Thirteen Days after the Beginning of the Experiment.—The controls and the larvæ of the iodine experiment do not show any changes, in particular no signs of metamorphosis. Every one of the three larvæ kept in iodothyrin was found this morning to have shed its skin for the first time.

These three larvæ absorbed the gills very quickly, moulted several more times in rapid succession, and 25 days after beginning of the experiment had reached the stage at which the larvæ must be removed from the water and put on land, in order to prevent

drowning. Fig. 3 shows No. 9 completely metamorphosed 134 days after the beginning of the experiment.

Forty-three Days after the Beginning of the Experiment.—Since 5 drops of iodine per 1,000 c.c. of water had no effect on metamorphosis, the iodine concentration was gradually increased and has reached today 9 drops in No. 5 and 8 drops per 1,000 c.c. of water in Nos. 4 and 6. Since it is believed that the alcohol used as solvent of the iodine might have some influence, too, on the animals, an equal number of drops of 95 per cent. alcohol is added from now on to the water in the controls.

Fifty-five Days After the Beginning of the Experiment.—The larvæ of the iodine series did not show any sign of an approach towards metamorphosis. Therefore the concentration of the iodine (and of the alcohol in the control series) was further increased and reached today 13 drops per 1,000 c.c. of water, a concentration of iodine more than 4 times the concentration sufficient to cause growth of the limbs in tadpoles.

Seventy-five Days After the Beginning of the Experiment.—The strength of the iodine solution was kept till today at 13 drops of a 1/20 M solution of iodine per 1,000 c.c. of water; in addition one larva (No. 5) was fed iodine crystals directly per mouth (1 crystal on the 57th, 63d, 68th, 70th and 74th day after the beginning of the experiment). Yet, in spite of these large amounts of inorganic iodine being adminstered to the larvæ, none of them shows any signs of metamorphosis so far.

From now on all larvæ, controls as well as experimentals, are kept in ordinary tap water, but the larvæ, Nos. 5 and 6 of the iodine series (No. 4 is used for a different experiment) are fed crystals directly per mouth.

One hundred and thirty-four Days After the Beginning of the Experiment.—The total number of iodine crystals fed to each one of the iodine-fed larvæ is now 25. This number of crystals was fed to No. 5 during a period of 76 days and to No. 6 during a period of 59 days. These large amounts of inorganic iodine were incapable of accelerating metamorphosis. In both the controls and the experimentals one larva has shed its skin, the iodine larva (No. 6) being now farther advanced than the corresponding control. But No. 5, the other iodine-fed larva, is completely

larval and distinctly less far developed than the least advanced control larva No. 2.

One larva of each set was photographed today. Fig. 1 shows the least advanced control larva No. 2, Fig. 2 the least advanced iodine-fed larva No. 5, and Fig. 3 the iodothyrine-fed animal No. 9. While the latter animal is a completely metamorphosed terrestric salamander and has been so for several months, both the control and the iodine-fed animal are larvæ. The control possesses, however, slightly shorter gills, its eyes are slightly more bulging than those of the iodine-fed larva (not visible in the photograph) and the ventral portion of the fin is greatly absorbed as compared to the completely preserved fin of the iodine-fed animal. On dissection the small intestine of the control, although the control larva was larger than the iodine-fed larva, was found to be shorter (308.5 mm.) than that of the iodine--fed larva (387 mm.).

In short 134 days after the beginning of the experiment the least advanced control larva proved to be nearer metamorphosis than the least advanced iodine-fed larva, although the latter was kept, till the 75th day after the beginning of the experiment, in iodine solutions of concentrations up to 13 drops of 1/20 M solution of inorganic iodine per 1,000 c.c. of water, and in addition had received a total number of 25 iodine crystals during the last 76 days of the experiment.

Discussion.

The experiments reported in this article fully confirm the experiments reported in a previous paper, in which I showed that inorganic iodine does not enforce metamorphosis of normal larvæ of the salamander *Ambystoma punctatum*.[5] The administration of inorganic iodine, even if this substance be administered in comparatively large doses, is incapable of bringing about the amphibian metamorphosis in those species in which the control of the excretion of the thyroid hormone is of such a kind that no hormone can escape from the thyroid before the end of the larval period.[5, 8]

[8] Uhlenhuth, E., *J. Gen. Physiol.*, 1919, i, 473–482, and *Am. Nat.*, 1921, lv, 193–221.

In the mind of the readers not quite familiar with the amphibian technique doubt may arise as to the correctness of the interpretation of the metamorphosis of one of the iodine-fed larvæ as not being due to the action of the iodine. I may mention, therefore that all of the axolotl larvæ, when kept under the conditions employed in the present control experiments, do undergo metamorphosis spontaneously. Consequently metamorphosis of the iodine-fed axlotl larvæ could be interpreted as the result of the iodine administration only, if it would have occurred at a greatly precocious date and simultaneously or nearly simultaneously in all the experimental larvæ. Not only were these conditions not fulfilled, but one of the experimental larvæ did not metamorphose at all and, at the termination of the experiment was farther away from the metamorphosis than any of the control larvæ, although observation extended over a period of more than 4 months, during which time large amounts of iodine were administered.

That the dosis of iodine was too small to enforce metamorphosis, can not be assumed; for, as will be discussed presently, the dosis of iodine administered to the larvæ of the iodine series was far in excess over the quantity of iodine contained in the dosis of iodothyrin which caused a very rapid metamorphosis.

Furthermore it is impossible to explain the ineffectiveness of the inorganic iodine by assuming that the larvæ employed in the iodine experiment had entered a stage at which the responsiveness to the stimuli causing metamorphosis has been lost. Not only could metamorphosis easily be produced by iodothyrin, but occurred spontaneously in the controls and in many other axolotl larvæ comparable to the iodine-fed larvæ in every respect (except for the iodine administration).

Hence it is evident that inorganic iodine is incapable of causing metamorphosis of the salamander larvæ.

Regarding the rôle of the iodine in the thyroid hormone these experiments also show, in a very striking manner, that the effect of the iodothyrin is not due to its iodine content. If it were due to its iodine content, quantities of inorganic iodine equal to those contained in an effective dosis of iodothyrin should cause metamorphosis. This expectation, however, is not fulfilled in the re-

sults of the present experiments. Bayer's iodothyrin, according to Bayer's "Materia Medica" and according to Baumann's own description of this substance,[9] contains 0.3 mgm. iodine per 1 gm. of substance. Consequently a dosis of 0.1 gm. of iodothyrin, which causes very rapid metamorphosis of the axolotl larvæ, contains 0.03 mgm. iodine per 1,000 c.c. of water. The weakest solution of inorganic iodine employed in the iodine experiment (5 drops of a M/20 iodine solution per 1,000 c.c. water) contained approximately 1.0 mgm. of iodine per 1,000 c.c. of water (1 drop being equal to about 0.2 mgm. of iodine), while the strongest solution contained even 2.6 mgm. iodine per 1,000 c.c. water. In addition to these quantities of inorganic iodine the larvæ of the iodine series received, directly per mouth, a total amount of 25 iodine crystals which equals approximately 40 mgm. of iodine, the average weight of one crystal being 1.6 mgm. Yet these quantities of inorganic iodine did not cause metamorphosis, although they were greatly in excess over the quantity of iodine contained in an effective dosis of iodothyrin. It is obvious that it is not the quantity of iodine contained in the iodothyrin, which produces the effectiveness of the latter substance.

Lenhart's experiments[10] showed that the effectiveness of thyroid gland in the amphibian metamorphosis of tadpoles increases with an increasing amount of iodine. These experiments are frequently quoted to demonstrate quantitatively that the active substance of the thyroid hormone in the amphibian metamorphosis is iodine. In the light of the present experiments this interpretation of Lenhart's experiments seems untenable. As pointed out in the introduction of this article, it is probable that the inorganic iodine does not produce the metamorphosis of tadpoles in its inorganic form, but after the thyroid apparatus has elaborated from it the thyroid hormone. Therefore Lenhart's experiments may be simply the expression of the fact that the tadpole thyroid, as claimed by Swingle[11] elaborates more hormone if more iodine is available. Moreover it is very probable that in Lenhart's experiments an increased amount of iodine contained

[9] Baumann, E., and Roos, E., *Zeitschr. f. physiol. Chem.*, 1895–96, 481–493.
[10] Lenhart, C. H., *J. Exper. Med.*, 1915, xxii, 739–746.
[11] Swingle, W. W., *J. Exper. Zoöl.*, 1919, xxvii, 417–425.

in the specimens of thyroid gland which were fed to the tadpoles corresponded to an increased amount of thyroid hormone (A-iodine, Kendall); in this case Lenhart's experiments would mean that metamorphosis is the more accelerated, the more thyroid hormone there is supplied to the tadpole, a result which corresponds with quantitative experiments on the action of iodothyrin in salamanders[5] and does not throw any light on the rôle of iodine in the thyroid hormone.

Swingle[12] in support of his viewpoint that inorganic iodine as such causes the amphibian metamorphosis, has emphasized the specificity of iodine, the administration of inorganic bromine being incapable of causing metamorphosis. It is not impossible, however, that this fact merely means that the organism does not possess a mechanism by means of which bromine can be employed in the manufacture of thyroid hormone; if it were possible to make synthetically, outside the organism, a substance identical with Kendall's thyroxin in every respect, but possessing a bromine atom in place of every iodine atom, this substance may be capable of producing metamorphosis.

Summary.

1. Iodothyrin and inorganic iodine, in known quantities, were administered to so-called axolotl larvæ of the salamander *Amblystoma tigrinum*.

2. A dosis of iodothyrin containing only 0.03 mgm. iodine per 1,000 c.c. of water caused metamorphosis 13 days after its first administration.

3. A dosis of inorganic iodine 33 to 86 times larger and feeding still larger doses directly per mouth did not cause metamorphosis.

4. The amphibian metamorphosis is truly the expression of the thyroid activity and not the result of the effect of inorganic iodine.

5. Inorganic iodine as such is not the active principle of the thyroid hormone.

[12] Swingle, W. W., *J. Gen. Physiol.*, 1919, i, 593–606.